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THE EFFECT OF SHORT CHAIN FATTY ACIDS ON THE INSULIN SENSITIVITY OF PIGS

BY

KARENA V. J. APPS

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for

THE DEGREE OF

MASTER OF SCIENCE

IN

NUTRITION

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SPRING, 1993

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THE EFFECT OF SHORT CHAIN FATTY ACIDS ON THE INSULIN SENSITIVITY OF PIGS

submitted by **Karena V. J. Apps** in partial fulfilment of the requirements for the degree of Master of Science in Nutrition.

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DATE: November 26, 1992

To: D.R. Eccles

You bring out the best in me.

Thank you.

Karena

ABSTRACT

High carbohydrate and high fibre diets have been reported to increase the sensitivity of peripheral tissues to glucose in both diabetic and non-diabetic human subjects. This phenomenon has been reported with both acute and long-term fibre supplementation. Short chain fatty acids have a glucose independent effect on plasma insulin concentrations of ruminants. It was hypothesized that high fibre diets are associated with increased short chain fatty acid production and absorption which improves glucose regulation.

To test this hypothesis, six pigs (20kg) were fitted with portal and jugular catheters. Animals received seven day experimental infusions of each of three individual short chain fatty acids (propionic, butyric and acetic acid) and a saline control through the portal catheter to deliver 0.01mmol SCFA/kg/min. Animals were fed a low fibre diet to minimize short chain fatty acid production from the large bowel fermentation of fibre. On the seventh day an intravenous glucose tolerance test was performed (445.05mg glucose/kg body weight) and jugular blood samples taken to determine glucose, insulin, glucagon and free fatty acids concentrations.

Short chain fatty acids infusions did not affect fasting levels of glucose, insulin, glucagon or free fatty acids ($p>0.05$). Glucose tolerance (K_G) and insulin sensitivity (S_I) were unaffected by short chain fatty acids ($p>0.05$). Glucagon concentrations were significantly different between propionic and butyric acid at 50 minutes post-glucose ($p<0.05$). The pig was seen to be a highly insulin sensitive animal. Insulin sensitivity decreased with increasing body weight in a manner similar to humans.

This thesis demonstrates that intravenous short chain fatty acid infusions have no effect on the insulin sensitivity or glucose tolerance of pigs.

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LIST OF ABBREVIATIONS

°C = degrees celsius

GI = glycemic index

SCFA = short chain fatty acid

IDDM = insulin dependent diabetes mellitus

NIDDM = non-insulin dependent diabetes mellitus

CHD = coronary heart disease

OGTT = oral glucose tolerance test

IVGTT = intra-venous glucose tolerance test

NEFA = non-esterified fatty acids

HCF = high carbohydrate/fibre

IST = insulin suppression test

FFA = free fatty acids

BMI = body mass index

S_I = insulin sensitivity (min^{-1}/uU/mL)

K_G = glucose tolerance (min^{-1})

p = probability

SEM = standard error of the mean

PUFA = polyunsaturated fatty acids

GIT = gastro-intestinal tract

TPN = total parental nutrition

SAS = statistical analysis system

BW = body weight

LITERATURE REVIEW

1 INTRODUCTION

During the last decade, the importance of dietary fibre in the North American diet has been emphasized by many groups (Canadian Diabetes Association, 1984; American Diabetes Association, 1986; American Heart Association, 1986; National Research Council (USA), 1982; Health and Welfare Canada, 1985). High fibre diets have been associated with lower incidence of several diseases and/or the management of some of these same chronic conditions and illnesses, including cardiovascular disease, obesity, diverticulitis, inflammatory bowel disease, colon cancer and diabetes (Anderson, 1986).

Diets which are high in both complex carbohydrates and dietary fibre are currently recommended for the dietary management of diabetes mellitus (National Nutrition Committee of the Canadian Diabetes Association, 1984). Human studies which examine the metabolic response to a glucose challenge have shown that high fibre supplemented diets flatten the glucose curve and can actually decrease fasting insulin and glucose levels (Miranda & Horwitz, 1978: Pastors et al, 1991:

Fukagawa et al, 1990). The ability of a high-fibre meal to modify the post-prandial glucose curve is a reflection of its glycemic potency.

Diets high in fibre have also been reported to improve the sensitivity of the body's peripheral tissues to insulin (Chen et al, 1990: Fukagawa et al, 1990), reducing the amount of insulin required to dispose of a glucose load. This characteristic of a high fibre diet may be important to both the diabetic and the non-diabetic individual. However, the mechanisms of action remain unknown.

2 DIETARY FIBRE

2.1 DEFINITION

The definition of dietary fibre continues to be controversial. There are predominately two approaches which are taken in attempt to reach a succinct denotation. Some prefer using a chemical definition whereas other prefer a physiological definition.

Dietary fibre is defined physiologically as the non-starch polysaccharide and lignin components of plants which cannot be digested by mammalian enzymes (Anderson, 1986).

Dietary fibre can be further divided into two categories, water-soluble and water-insoluble (Meyer et al, 1988). Soluble fibres tend to be more rapidly and completely fermented in the large intestine and include such compounds as pectins, gums and some hemicelluloses (Anderson, 1986). Examples of food sources of soluble fibre are fruits, oats, and legumes. Insoluble fibres tend to be less fermentable and are primarily composed of cellulose, hemicellulose and lignin. Food sources of insoluble fibre are cereal grains. The properties of fibre change as they move throughout the gastrointestinal tract (GIT) so it is difficult to predict their physiological effects from foods (McBurney et al, 1985).

The chemical definition is preferred by some because it is more precise in defining the composition of dietary fibres. To define fibre chemically it is necessary to determine the individual monosaccharides in the non-starch polysaccharide (Englyst & Cummings, 1984). However, this definition does not account for any physiochemical properties.

There are two forms of starch, available starch and resistant starch. Available starch can be completely degraded by digestive enzymes and is therefore not a consideration when discussing dietary fibre. Starch can be

deemed resistant due to three factors; physical structure, densely packed granules, and retrogradation from cooking or processing (Hollenbeck & Coulson, 1991). Resistant starch is unchanged by digestive enzymes in the small intestine and is therefore moved into the colon where it is fermented along with non-starch polysaccharides (Stephens, 1993). Resistant starch is considered by some to be included in the definition of dietary fibre (Asp et al, 1988).

2.2 LARGE BOWEL FERMENTATION

A high intake of dietary fibre has a positive contribution to the overall health and integrity of the gut (Cummings, 1981). A high intake of insoluble fibre increases fecal bulk. This increase in fecal mass can decrease the occurrence of constipation which can contribute to haemorrhoids and diverticular disease in the colon (Anderson, 1986). The transit time of high fibre digesta through the large intestine is longer (20-40 hours) than through the stomach and small intestine (2-16 hours) (Low, 1985). The most significant consequence of a longer transit time is the prolific growth of colonic bacteria.

Several gastro-intestinal changes occur when a diet is changed from one of low fibre content to one of high fibre.

Because of the increase in fermentable substrate, microbial growth and proliferation increases as does intestinal mass (McBurney & Van Soest, 1991). This may be due in part to the increased availability of preferred fuel. These morphological changes may impact the body's response and handling of all nutrients including dietary fibre.

Fibre is fermented by colonic bacteria which produce short chain fatty acids (SCFA), H_2, CO_2, methane and H_2O. The SCFA found in the highest colonic proportions are acetate, propionate and butyrate (Cummings et al, 1986). These three SCFA appear in an almost constant molar ratio of 60:25:15 and represent 83% of the SCFA produced in the colon (Settle, 1988; Mortensen et al, 1991). Smaller levels of isobutyrate, valeric, isovaleric, caproic and isocaproic acids are also seen (Bugaut, 1987). Acetate (2 carbon chain) is largely metabolized in the distal colon where it is converted to glutamate and aspartate (Marty et al, 1985). Propionic acid is an odd-chain (3C) fatty acid and is seen to be glycogenic in ruminants and other mammals (Bugaut, 1987; Demigne et al, 1990). Butyrate, a four carbon chain, is metabolized by colonocytes to CO_2, and the ketone bodies beta-hydroxybutyrate and acetoacetate (Roediger, 1980). SCFA are absorbed in the jejunum, ileum, colon and rectum (Hoverstad, 1986) with the rate of absorption being greatest in the colon. SCFA have been predicted to provide between

3-11% of metabolizable energy intake of humans (McBurney & Thompson, 1991).

The metabolism of butyrate by the colonic mucosa stimulates an increase in colonic cell mass (Roediger, 1980). Roediger (1980) measured the fuel consumption of human colonic mucosa in vitro and reported that 70% of oxygen consumed by the ascending and descending colon could be attributed to the oxidation of butyrate. Ketogenesis also occurs in the mucosal cells, and incorporates a portion of the butyrate produced by fibre fermentation. Ketone bodies also serve as a fuel for human colonic mucosa (Roediger, 1980). Ketone body production is greater in the proximal than the distal colon (Roediger, 1980). Of the papers reviewed by Settle (1988), the order of preference by the colonic mucosa (determined by in vitro studies) were found to be butyrate > acetoacetate > L-glutamine > D-glucose.

Using an in vitro system, Scheppach et al (1992) found propionate (25mmol/L) and butyrate (10mmol/L) to stimulate human colonocyte crypt proliferation to the same degree as the physiological mix of acetate (60mmol/L), propionic (25mmol/L) and butyrate (10 mmol/L). The authors concluded that butyrate was highly effective at stimulating crypt proliferation whereas propionic had a smaller stimulatory effect and the impact of acetate was minor.

Fleming et al (1991b) studied SCFA utilization by rat colonocytes and found that the mucosal cells utilized SCFA (5mmol/L eqimolar concentrations) in the following order: butyrate > acetate > propionate. It is evident that there still remains some uncertainty in the role that each SCFA plays in the proliferation and growth of the intestinal mucosa, but butyrate appears to be the primary and preferred SCFA fuel of the gut epithelium.

Current theory holds that butyrate also stimulates the proliferation of the mucosal cells of the small intestine (Demigne & Remesy, 1985). The order of fuel preference of the small intestine is L-glutamine > ketone bodies > D-glucose (Windmueller et al, 1978). Rombeau (1988) reported a trophic effect on the small intestine of rats seen during colonic infusions of SCFA at physiological concentrations. The importance of SCFA as a fuel to the small bowel is not yet defined.

The SCFA which are not metabolized in the colonic mucosa enter the portal vein. The clearance of SCFA by the liver is very efficient. The systemic concentrations of acetate is 3-4 times lower than portal concentrations whereas propionic and butyrate concentrations are 10 times lower (Dankert et al, 1981; Yen et al, 1989).

SCFA affect the microcirculatory capacity of the colon. Mortensen et al (1991) showed that SCFA used alone or in combination dilate the arteries surrounding the colon in a concentration dependent manner. The authors offered two explanations for this phenomenon. Firstly, the increase in tissue mass due to the growth of the mucosal and interstitial cells demanded a need for an increase in blood flow. Secondly, cell metabolism increased in response to increased availability of fuel. Mortensen et al (1991) also observed angiogenesis in the enlarged colon and concluded that SCFA also initiated an increase in the number of total capillaries in the colon.

2.2.1 PRODUCTION OF SHORT CHAIN FATTY ACIDS

The amount of fermentable carbohydrate (ie: fibre and resistant starch) consumed in a day will be the primary determinant of SCFA production in the colon. Methods for determining total colonic production of SCFA are not quantitatively accurate. There are three methods currently used to estimate SCFA production in monogastrics. These assume that the fermentation processes, substrates used and metabolites produced are similar to those measured in ruminant fermentation and in vitro studies.

The first method uses an equation developed by Miller and Wolin (1979). This equation is based on the molar ratios of SCFA in the feces and known quantities of CO_2 and methane, using the following equation:

$$34.5\ C_6H_{12}O_6 \text{ ----> } 64\ \text{SCFA} + 23.75\ CH_4 + 34.23\ CO_2 + 10.5\ H_2O$$

Cummings (1981) estimated that human colonic bacteria ferment approximately 20g of carbohydrate/day. Of this total, 15g are from dietary fibre and 5g are from soluble carbohydrate. Therefore, using the equation of Miller & Wolin (1979), 20g of fermented carbohydrate would yield approximately 200mmol of SCFA/day.

The second method used to estimate daily SCFA production is based on fecal bacterial excretion (Cummings, 1981). Smith and Bryant (1979) determined that the production of 62g of bacteria cells requires the fermentation of approximately 1 mole of hexose. Smith and Bryant (1979) also suggested that the amount of bacteria produced in the human gut is equivalent to 13% of the weight of the carbohydrate fermented in the gut. However, this is a very rough estimate since the amount of bacterial cells produced/g hexose fermented largely depends on the presence and availability of preformed monomers (eg; amino acids)

(Cummings, 1981). Other factors such as cell wall composition, large bowel retention time and energy requirements (variable among species) of the bacteria will also contribute to the margin of error when using this methodology.

A third way to determine the amounts of SCFA produced in the colon is by in vitro fermentation of ileal effluent (McBurney & Thompson, 1989). Samples of freeze-dried effluent are inoculated with fresh feces and allowed to incubate in anaerobic conditions for 24 hours. This time frame represents the average colonic retention time. The supernatant produced from this intubation is analysized using liquid gas chromatography to determine SCFA production in the human colon (McBurney & Thompson, 1989; McBurney & Sauer, unpublished data).

In summary, the methods for estimating the amount of SCFA produced in the human colon are estimates only and subject to error. For example, it is difficult to accurately measure the amount substrate reaching the colon daily, the bacterial mass within the colon or the energy requirements of these bacteria. As a result, current estimates of SCFA production from feeding studies in humans are approximations only.

2.2.2 ABSORPTION

The human absorption rate of SCFA of 6-13 $\mu mol/cm^2/hr$ is comparable to that measured in other animals (Cummings, 1981). The absorption of SCFA is invariably linked with the disappearance of bicarbonate. Bicarbonate appears in the colon in a constant ratio with the disappearance of SCFA, and does so independently of the colonic chloride/HCO_3^- exchange (Fleming et al, 1991a). This relieves the lumen acidity and maintains the acidity of the colon close to pH 6 (McBurney & Van Soest, 1991). On a molar basis, HCO_3^- appearance is equal to approximately 50% of the total SCFA absorbed (Cummings, 1981). One proposed mechanism for SCFA absorption involves the hydration of CO_2 in the lumen of the gut. This would allow the protonation of the SCFA and its rapid absorption in an ionized form (Cummings, 1981). This mechanism would also explain the accumulation of HCO_3^-.

However, sodium absorption also increases during the absorption of SCFA. The hypothesized interaction between SCFA and Na^+ is through the recycling of the hydrogen ion. If SCFA are absorbed in an ionized form, this might drive the Na^+/H^+ exchange which would stimulate Na absorption (Cummings, 1981).

One of the factors which makes the transport of SCFA

across the gut wall difficult to measure is that a large percentage of the acids are metabolized by the epithelial cells. Thirty percent of acetate (Stevens & Stettler, 1966) and 70-80% of butyrate produced (Stevens & Stettler, 1966: Roediger, 1980) appear as the ketone bodies acetoacetate and beta-hydroxy-butyrate in the portal blood.

3 DIABETES MELLITUS

The significance of the role of dietary fibre in diabetes remains unclear. It has been hypothesized that a fibre depleted diet may be a causative factor in the clinical onset of diabetes mellitus (Miranda & Horwitz, 1978; Trowell, 1975). However, the majority of research linking fibre and diabetes has focused on the dietary management of both insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM).

Diabetes mellitus is a disease of the endocrine system. It is most commonly manifested in one of two ways. The first is IDDM, when there is diminished secretion of insulin from the beta cells of the islets of Langerhans and therefore insufficient insulin secretion to maintain normal blood glucose concentrations post-prandially or during the fasted state. Management requires the coordination of exogenous insulin and dietary mangement. The result of poor

IDDM management is dangerously high or low blood glucose levels which can lead to coma and eventual death. The second manifestation of diabetes is NIDDM. The primary cause of NIDDM is the development of insulin resistance in peripheral tissues. The presence of insulin resistance in conjunction with degeneration of the beta-cells will present a diabetic condition.

Both NIDDM and IDDM may be compounded by a decrease in the peripheral tissue's sensitivity to insulin (Olefsky, et al, 1973). With this decrease in insulin sensitivity, the extrahepatic-tissues do not respond to the insulin signal and therefore do not transport glucose from the blood into the tissues. In some cases of NIDDM, the beta-cell is actually producing normal amounts or an excess of insulin but the tissues are insensitive to it. Three factors are believed to decrease insulin sensitivity: 1) increasing age (Chen et al, 1985), 2) obesity (Bergman et al, 1981b) and 3) diet (Fukagawa et al, 1990). A diabetic condition can arise from one or a combination of any of these metabolic changes.

Initially dietary recommendations for diabetic patients emphasized low levels of carbohydrate to maintain low post-prandial blood glucose concentrations (Anderson & Ward, 1979). Recently, diabetic patients have been advised to consume high levels of complex carbohydrates and plant

fibres (National Nutrition Committee of the Canadian Diabetes Association, 1984) because research indicates that the addition of fibre to the diabetic diet is accompanied by a reduction in post-prandial hyperglycemia (Anderson & Ward, 1979; Jenkins et al, 1976, 1977). Diabetic individuals are at increased risk of coronary heart disease (CHD) (Guidelines for the Nutritional Management of Diabetes Mellitus: A Special Report, 1984; Canadian Diabetes Association). Therefore the present dietary recommendations also accommodate the dietary guidelines for patients with CHD.

4 INSULIN SENSITIVITY

4.1 Methods of Determination

The body's ability to dispose of dietary carbohydrate depends on two distinct mechanisms. The first is the pancreatic beta cell secretion of insulin stimulated mainly by changes in peripheral glucose concentration. The second is the sensitivity of the peripheral tissues to the secreted insulin (Bergman et al, 1981a). It is this second mechanism of insulin resistance which is thought to be, in addition to relative impairment of pancreatic insulin secretions, an important etiological factor in the development of NIDDM

(Bergman & Bowden, 1981). Insulin resistance exists when a given and known quantity of insulin produces less than the expected biological effect (Olefky, 1981). The body's sensitivity to insulin can be determined by measuring its ability to dispose of an orally or intravenously administered carbohydrate. The oral glucose tolerance test is the most commonly recommended method of determining metabolic glucose/insulin abnormalities. By measuring both plasma glucose and insulin levels, it is possible to derive a mathematical measure of insulin sensitivity when a intravenous glucose tolerance test is performed. Insulin sensitivity can therefore be defined as the effect of insulin to augment glucose's ability to reduce its own plasma level (Finegood et al, 1984).

Insulin sensitivity has been historically difficult to measure in an intact organism (Finegood et al, 1984) because it required pharmacological intervention. During glucose disposal the beta cells and tissues are in continual communication. Therefore changes in the secretion of insulin will be reflected quickly in the glucose concentration of the blood; conversely, endogenous glucose production will invoke a rapid secretion of insulin from the beta cells (Bergman et al, 1985). This tight feedback loop makes it difficult to draw any conclusions regarding insulin sensitivity from the OGTT or IVGTT alone. As a result, two methods have been

developed to open the glucose/insulin feedback loop and determine insulin sensitivity. They are the insulin suppression test and the glucose clamp.

4.1.1 INSULIN SUPPRESSION TEST

The insulin suppression test (IST) was developed at Stanford University and is based on the inhibition of pancreatic insulin while exogenous glucose (6 mg/min/kg) and insulin (maintained at .48pmol/min) are introduced into the bloodstream (Bergman et al, 1985). Endogenous insulin is suppressed by infusion of epinephrine (6 ug/min), somatostatin (125-250 ug/hour) or a beta-adrenergic blocker (.08 mg/min for 150 min). Insulin resistance is determined by assuming that the level of serum glucose increases as the tissue resistance to insulin increases.

4.1.2 THE GLUCOSE CLAMP

The glucose clamp method of determining insulin sensitivity was developed at Baltimore City Hospital in 1962 (Bergman et al, 1985). When insulin is administered exogenously, the body's utilization of glucose increases as endogenous glucose production decreases. This results in an

overall decrease in the plasma concentration of glucose. The clamp technique requires the replacement of the glucose lost in this declining hepatic production and the glucose utilized by the tissues (Bergman et al, 1985). By maintaining a constant concentration of plasma glucose, one has determined the net effect of insulin to alter the body's production and utilization of glucose. There are many methodological approaches to performing a clamp. A general protocol starts with a basal sampling of blood. Then insulin is administered either by injection plus an infusion or by infusion alone. Blood is then sampled at frequent intervals and the required amount of glucose is calculated and infused so that a steady state is reached and maintained. The rate of glucose infusion can be considered an indication of insulin action.

There are methodological problems involved with both of these closed loop techniques of determining insulin sensitivity and these have resulted in a good deal of variance in results. For example, it is very difficult to achieve a steady state of glucose and/or insulin with the insulin suppression test. If a steady state is not maintained then the accuracy of the data derived is in question. Another problem common to both of these methods is that they do not take into consideration the non-insulin dependent utilization of glucose. As a result, the use of these methods is rarely found outside the research laboratory

(Finegood et al, 1984). Consequently, a physiological method has been widely accepted to calculate insulin sensitivity. This method, the Minimal Model Method, differs from the previous two reviewed methods of determining insulin sensitivity because it is a closed loop method. This means that there is no exogenous control of insulin or glucose. This method requires only that the plasma insulin and glucose levels be determined during an intravenous glucose tolerance test (IVGTT). The minimal model infers insulin sensitivity using an iterative computer program. This is the simplest and least invasive method of determining insulin sensitivity. The computer model simulates plasma glucose changes if the changes in plasma insulin are provided (Bergman et al, 1985).

The minimal model defines two characteristics of glucose kinetics. The first parameter is S_G, the glucose effectiveness independent of an increment in insulin. S_G is a measure of the ability of glucose to enhance its own disappearance from plasma at basal insulin concentrations and has the units $min^{-1}/\mu U/mL$ (Bergman et al, 1986). The second parameter is S_I, which is an index of insulin sensitivity (Bergman et al, 1986). S_I is a measure of the ability of the increase in plasma insulin to enhance glucose's ability to accelerate its disappearance from plasma and has the units min^{-1}.

4.2 FACTORS INFLUENCING INSULIN SENSITIVITY

4.2.1 OBESITY

Many factors affect an individual's sensitivity to insulin. Obesity is one of the physiological factors which has been shown to decrease insulin sensitivity. Bergman et al (1981b) examined the pancreatic response and insulin sensitivity of both lean and obese subjects using the minimal model method. Subjects were divided into four groups based on glucose tolerance: 1)obese:good tolerance, 2)obese:poor tolerance, 3)lean:good tolerance and 4)lean:poor tolerance. Low tolerance in the lean group was attributed to insufficient pancreatic response to a glucose load. There were no differences observed in tissue sensitivity between the two lean groups (Bergman et al, 1981b). In the obese subjects however, low tolerance was related to decreased insulin sensitivity. It was determined that insulin sensitivity was decreased by 60% in the obese:low tolerance group compared to the lean:good tolerance group (Bergman et al, 1981b).

The insulin resistance characteristically seen with obesity involves both the oxidative and non-oxidative pathways of glucose metabolism (Franssila-Kallunki et al,

1992). In obese individuals, the increased supply of free fatty acids (FFA) could increase the oxidation of lipids through the activation of the glucose-FFA cycle. Fatty acids and glycerol are released from both muscle and adipose depots to be re-esterified into glycerides, oxidised, or transferred to plasma albumin (Randle et al, 1963). The uptake of glucose from blood by adipose tissue inhibits the movement of FFA from adipose to muscle through the blood stream (Figure I-1). Consequently, FFA concentrations increase in the plasma which inhibits the uptake of glucose into muscle tissue (Figure I-2). This cycle provides a simplistic mechanism of the balance between glucose and FFA which is independent of hormonal control.

Insulin modifies the balance of this cycle by enhancing muscle adipose uptake of glucose and therefore inhibiting the release of fatty acids from adipose tissue and increasing the esterification of FFA (Randle et al, 1963). Based on this cycle, Randle et al (1963b) proposed that high levels of FFA are involved in the development of NIDDM.

Following Randle's proposed cycle, Gomez et al, (1972) studied whether changes in glucose tolerance could result from competition between the oxidation of glucose and free fatty acids. The infusion of lipids into healthy, normal weight subjects showed an impairment in glucose oxidation and

FIGURE I-1

Glucose-Free Fatty Acid Cycle

Blood Phase

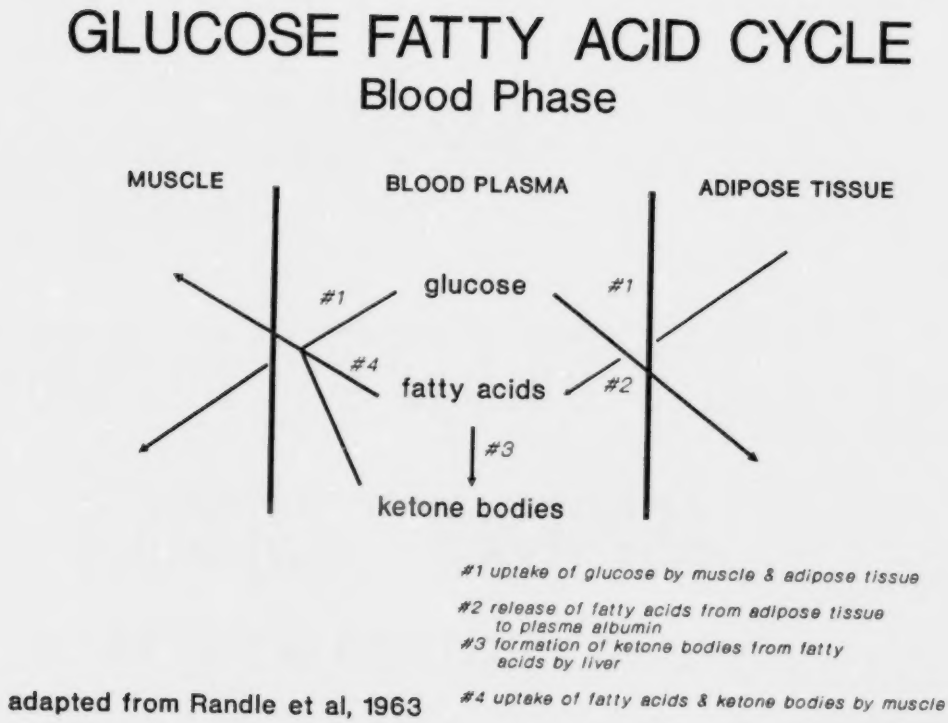
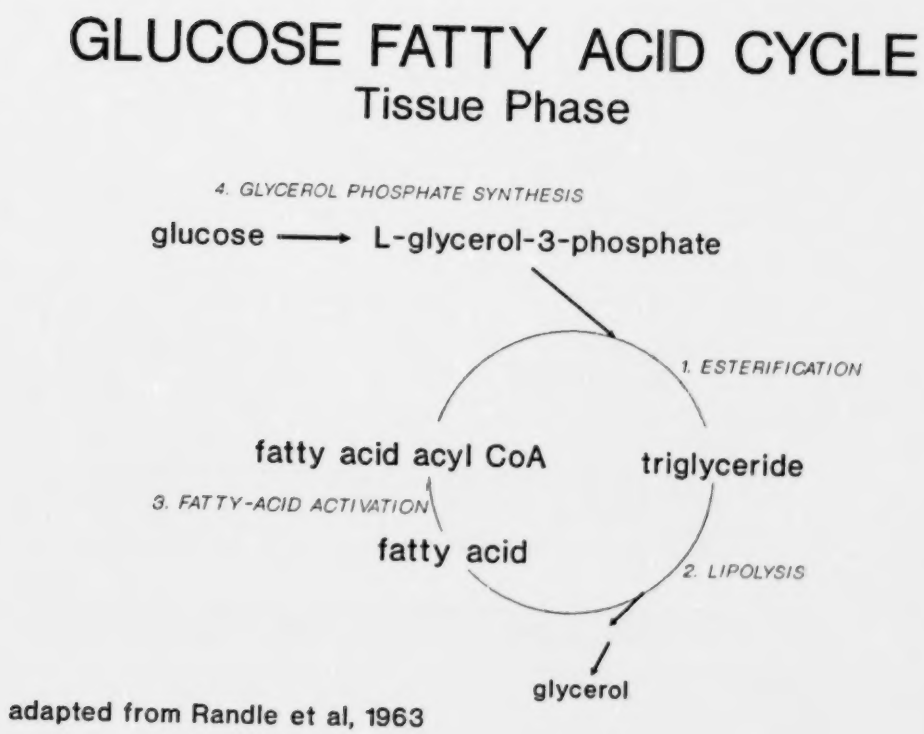


FIGURE I-2

Glucose-Free Fatty Acid Cycle

Tissue Phase



adapted from Randle et al, 1963

an increase in lipid oxidation despite an increased insulin response to a glucose challenge. Gomez et al (1972) concluded that the entry of FFA into muscle tissue is dependent on plasma concentration and is oxidized in preference to glucose.

The principle of FFA and glucose competition was again tested by Franssila-Kallunki et al (1992). Glucose metabolism was examined in twelve obese subjects (mean BMI: 33.4 ± 1.1) and again after a six week intensive weight loss period (mean BMI: 29.9 ± 1.0). The weight that was lost was determined to be 85% fat mass. The reduction in weight produced the following results compared to the non-obese control subjects: insulin-stimulated glucose metabolism was increased but not normalized; basal rate of glucose oxidation was increased to normal levels; insulin-stimulated glucose oxidation was enhanced; the rate of non-oxidative glucose metabolism was reduced to normal levels (Franssila-Kallunki et al, 1992). The rate of insulin-stimulated, non-oxidative glucose metabolism was reduced by 40% in the obese individuals compared with the control subjects (Franssila-Kallunli et al, 1992). Fasting insulin levels dropped from 137 ± 22pmol/L before weight loss to 79 ± 14pmol/L after weight loss. The authors concluded from these results that the operative glucose-FFA cycle contributes to insulin

resistance and that the reversal of this cycle can reduce resistance. In addition, improved insulin sensitivity was linked to reduced basal and post-prandial insulin concentrations (Franssila-Kallunli et al, 1992).

The examination of the consequence of elevated plasma FFA on glucose production and utilization was undertaken by Ferrannini et al (1983). This study used 52 healthy subjects who were within 20% of their ideal weight. Three different protocols were used in three different groups: (A) hyperinsulinemia with euglycemic (plasma insulin concentration 600pmol/L and fasting levels of plasma glucose), (B) hyperinsulinemia with hyperglycemia (plasma glucose 7mmol above fasting levels, 1.8pmol/min kg insulin), and (C) hyperglycemia with basal insulin levels (maintained at 11.2mmol above fasting levels). These protocols were repeated twice, once involving a Intralipid infusion along with the above detailed protocol.

The results of protocol A showed that while holding plasma glucose constant FFA levels fell rapidly from a mean basal levels of .48 ± .10μmol/mL to .15 ± .03μmol/mL (Ferrannini et al, 1983). During the lipid infusion, the total glucose uptake was significantly lower (.35 ± .07 vs .41 ± .3mmol) than the control study.

When the results of the hyperinsulinemia protocol were examined with hyperglycemia, plasma FFA levels fell from .55 ± .08μmol/L to .15 ± .03μmol/L and the total glucose uptake was stimulated. Glucose uptake was impaired by lipid infusion when compared to the control trial (.35 ± .06mmol kg vs .55 ± .06mmol kg), regardless of the elevated insulin levels (Ferrannini et al, 1983). Glucose production was also seen to be suppressed during the 2nd hour of the test in both the control and lipid infusion trials of this protocol.

The final protocol examined in this study showed that hyperglycemia was associated with insulin levels similar to basal values and that FFA concentrations rose slightly at the 40-60 min mark and then returned to baseline. Glucose uptake only rose 36 ± 10% during this test, however, the amount of glucose lost in the urine increased significantly (Ferrannini et al, 1983). During the lipid infusion, FFA levels rose modestly whereas all other measurements remained at levels seen in the control test. Basal glucose output (.11 ± .001 mmol/min kg) was suppressed 82 ± 22% in the control trial but during the lipid test endogenous glucose production dropped only slightly (.78 ± .03mmol/min kg vs .10 ± .001mmol/min kg). Therefore, the infusion of FFA caused a threefold increase in endogenous glucose production in the second hour of the study (Ferrannini et al, 1983).

When the data from the three protocols were pooled, a highly significant direct correlation between the degree of inhibition caused by increased plasma FFA concentrations and the individual rates of total glucose uptake was seen. If the results of this short term study were to be extrapolated to chronic metabolic states, it could be concluded that elevated concentrations of FFA can induce glucose intolerance if the increased FFA persist in the face of an insulin response that directs glucose flux into insulin sensitive tissue (ie: muscle) (Ferrannini et al, 1983).

Insulin-stimulated glucose uptake can be impaired in NIDDM patients (DeFronzo et al, 1982). The insulin regulation of FFA is less documented. Abnormal response of FFA in NIDDM patients are typically only seen in those who are obese (Franze et al, 1985). Franze et al (1985) hypothesized that FFA metabolism is normally regulated in non-obese NIDDM patients and therefore, plasma FFA levels would be similar to normal individuals while their plasma glucose levels would be disparate. The study group of 15 non-obese NIDDM patients and 15 normal subjects were admitted to Stanford General Clinical Research Centre and fed a diet composed of 17% protein, 40% fat and 43% carbohydrate. After three days plasma glucose, insulin and FFA levels were measured in fasted and fed states. Fasting and post-prandial glucose levels were higher in NIDDM versus normal subjects.

The insulin curves between the normal subjects and the NIDDM subjects were significantly different, however, when looking for interaction between time and group at each individual time point, were non-significant. The insulin levels of the NIDDM subjects were never lower than the normal subjects (Franze et al, 1985). FFA curves were examined in a similar fashion to insulin. The levels of FFA as an entity, were significantly higher in NIDDM patients. In contrast to the insulin results, significant interaction between time and group were observed.

The NIDDM subjects were divided into three groups based on degree of hyperglycemia. The results of this analysis show that the greater the degree of hyperglycemia, the higher the plasma FFA levels (Franze et al, 1985). The post-prandial insulin levels were seen to fall and the FFA concentrations rose as the magnitude of hyperglycemia increased. From the results of this study, it seems reasonable to conclude along with the authors that insulin is not able to regulate plasma FFA concentrations normally in non-obese NIDDM patients (Franze et al, 1985).

From the studies examined, it appears clearly that there is an interaction or feedback between plasma levels of FFA and glucose which is independent of insulin concentrations.

4.2.2 AGE

Insulin sensitivity seems to decrease with aging. Chen et al (1985) reported no significant differences between young and old men of similar weight in fasting glucose (5.1 ± .11 & 5.2 ± .06 mmol respectively) or insulin (48 ± 6 & 60 ± 6 pmol respectively) levels although the older men were more glucose intolerant after an OGTT challenge (7.3 ± .45 vs 6.0 ± .50 mmol) (Chen et al, 1985). Insulin response did not differ between the two age groups and therefore tissue insensitivity to insulin was concluded to have accounted for the relative glucose intolerance in the older group. The minimal model showed the insulin sensitivity of the young group (6.5 ± .6 $min^{-1}/\mu U/mL$) to be reduced 63% in the older group (Chen et al, 1985).

Coppack et al (1990) followed a large group (103) of newly diagnosed NIDDM subjects for a period of 10 years and monitored their glucose tolerance. The diabetic management of these subjects was seen to improve during the first year, whereafter there were significant increases in fasting insulin and glucose levels and insulin resistance, without a significant change in beta-cell function (Coppack et al, 1990). The increase in the age 53.5 years (range 46.2-60.7 years) to 63.5 years of the subjects was considered to have contributed to the deterioration of their diabetic condition.

4.2.3 DIET

Diet is a third factor which has been shown to impact peripheral sensitivity to insulin. Lowered levels of basal glucose and insulin concentrations were seen in healthy individuals when fed high fibre, high carbohydrate diets (Anderson & Chen, 1979; Villaume et al, 1984). Currently, diabetic individuals are recommended to consume diets which are high in complex carbohydrates and high in fibre because of apparent glucose lowering effects even though mechanisms are not clear. Fukagawa et al (1990) hypothesized that high carbohydrate and fibre diets would improve insulin sensitivity but that this effect may be age dependent. A group of young men (18-24yrs) and older men and women (67-86yrs) were studied. The overall average glucose disposal increased after 21-28 days on the test diet (Fukagawa et al, 1990). In examining the two groups separately, the younger group showed a significant increase in glucose disposal on the high carbohydrate and fibre diet, whereas the increase in the older group did not reach significance. The researchers concluded that high carbohydrate high fibre diets could significantly increase insulin sensitivity to physiological concentrations of insulin in healthy young men and they suggested an extrahepatic effect of the high fibre diet because this

response occurred in the absence of changes in the regulation of hepatic glucose output. (Fukagawa et al, 1990). Fasting insulin, glucose and cholesterol levels were also lowered on the high carbohydrate and fibre diet.

4.2.3.1 SMALL INTESTINAL EFFECTS OF FIBRE

Soluble fibres like pectin and guar are very viscous and delay gastric emptying, slowing the transit time of digesta in the small intestine (Meyer et al, 1988). Meyer et al (1988) found viscosity to be one mechanism whereby the emptying of the stomach was slowed. Glucose absorption was prolonged by the more viscous fibre, guar, than it was for pectin (Meyer et al, 1988). Fibre may also decrease the availability of both macro (proteins, fats, glucose) and micro-nutrients (vitamins, minerals) (Anderson, 1991) despite a longer transit time through the small intestine. However, with the consumption of a diet adequate in all nutrients, any nutrient absorptional losses due to fibre are minimal (Anderson, 1986). The action of digestive enzymes, gastrointestinal and pancreatic hormones may also be delayed by increased viscosity of the digesta or the physical presence of soluble fibre (Anderson, 1986); however this delay is believed to have a negligible impact on digestion.

Jenkins et al (1976, 1977) demonstrated that dietary fibre supplementations of guar and pectin decreased post-prandial blood glucose and insulin responses in diabetic and normal subjects. These authors hypothesized that insulin secretion or insulin sensitivity was influenced by soluble fibre intake.

Subsequently, Jenkins (1980) fed subjects two glucose drinks at 4 hours intervals with one of the tests containing 22.3 g of guar. An oral glucose tolerance test was conducted after both glucose drinks. Guar supplementation resulted in a significantly flattened glucose curve (Jenkins et al, 1980) but the changes were not related with increased insulin levels. The authors suggested that guar gum slowed the absorption of glucose resulting in reduced insulin secretion and post-prandial glycemia which stimulated a metabolic counter-regulatory response (Jenkins et al, 1980). They suggested that the initiation of this counter-regulatory response would result in increased levels of ketone bodies and non-esterified fatty acids (NEFA). Indeed, both ketone bodies and NEFA increased when the glucose load was given without the guar supplement. The authors suggested that both ketone bodies and NEFA could possibly be used as markers of the metabolic effects of fibre. Jenkins et al (1980) concluded that certain dietary fibres have the ability to improve glucose tolerance over a period of several hours.

Jenkin's (1980) examined prolonged metabolic changes linked with the feeding of high fibre meals by introducing the fibre supplement in a breakfast meal and examining glucose tolerance at lunch. The results of this work showed clearly that the addition of guar to the first of two drinks markedly reduced the rise in blood glucose of the second drink taken 4 hours later (Jenkins, 1980). The impact of the glucose concentration was non-insulin dependent because isulin levels did not change with the addition of guar.

Pastors et al (1991) explored the effect of psyllium fibre on post-prandial blood glucose and insulin levels immediately and up to 5 hours after the consumption of psyllium. The fibre source was ingested both at breakfast and supper. Peak post-prandial glucose concentrations were significantly decreased after both meals when psyllium was eaten. The decrease was greater after the evening meal. A significant decrease in serum insulin was also seen after ingesting psyllium relative to the control diet (Pastors et al, 1991). The effect of the fibre supplement on second meal OGTT was also examined. The fibre eaten at breakfast was seen to lower glucose and insulin levels at lunch. Peak glucose concentrations were 31% lower after lunch when a fibre supplement was taken at breakfast. The authors proposed two mechanisms by which psyllium caused an improvement in postprandial glucose tolerance. The first, in

agreement with previous research, was that psyllium forms a viscous gel, similar to guar, and delays the absorption of glucose by the intestinal epithelium. This mechanism also incorporates theories suggested in earlier studies whereby the gel slowed gastric emptying and/or the action of digestive enzymes. The second proposed mechanism was that fibre plays an additional metabolic role in its effects on glucose post-prandially. Psyllium evoked a smaller insulin response immediately after a meal which would be matched with a corresponding decrease in the counter-regulatory response and a smaller glucose basal undershoot. This results in a less severe counter-regulatory production of ketone bodies and free fatty acids. The final outcome of lower glucose, insulin, ketone bodies and free fatty acid levels, is an increase in insulin sensitivity. This would in turn increase potential for improved diabetic control (Jenkins et al, 1980; Pastors et al, 1991).

4.2.3.2 COLONIC EFFECTS OF FIBRE

The small intestinal effects on glucose absorption and metabolism have been well demonstrated (Jenkins et al, 1976, 1977; Anderson & Ward, 1979; Sator et al, 1981; Braaten et al, 1991). However, to explain metabolic changes

occurring 12-24 hours post-prandially, one must examine the effect of fibre in the large intestine.

Wolever et al (1988) fed healthy subjects test meals of different glycemic indices and different amounts of fibre the night before an OGTT and found that high fibre meals with a low glycemic index reduced the post-prandial rise in blood glucose after an overnight fast. Ketone body and NEFA concentrations were not affected by taking fibre supplement the night before an OGTT (Wolever et al, 1988). These results provide evidence that fibre may have prolonged (12-18 hours) metabolic effects on glucose tolerance which cannot be explained by the presence of fibre in the small intestine.

Kiehm et al (1976) reported that glucose tolerance of diabetic subjects significantly improved while on a diet consisting of 75% carbohydrate (414g: 114 g oligosaccharides and remainder polysaccharides) for two weeks. Compared to an ADA recommended diet composed of 43% calories as carbohydrate (234g: 109g oligosaccharides and the remainder polysaccharides). Indeed, some of the subjects had to reduce their insulin or sulphonylureas levels to properly manage their blood glucose levels while on the high carbohydrate diet.

In a similar study, using a 10 day experimental period, Miranda & Horwitz (1978) reported decreased fasting blood glucose levels in 7 of their 8 IDDM subjects when consuming an iso-carbohydrate diet containing 20 grams crude fibre. The patients with the greatest percentage decrease on the high fibre diet were those with the highest glucose levels on the low fibre diet (3 grams of fibre) (Miranda & Horwitz, 1978). Insulin levels were unaffected by diets because exogenous insulin dosages were not adjusted. Glucagon levels were significantly lower on the high fibre diets. The authors concluded IDDM patients can achieve lower blood glucose levels on high fibre diets without increasing their insulin dosage (Miranda & Horwitz, 1978).

Simpson et al (1979) fed IDDM patients a high carbohydrate high fibre diet for three weeks and found that exogenous insulin doses could be decreased, fasting glucose levels were lowered and glucose disposal following a an oral glucose challenge was improved. The study was repeated again after six weeks to reveal similar results.

The effect of high fibre and high carbohydrate diet combinations were examined relative to the ADA recommended control diet by Anderson and Ward (1979). The outcome of this 15 day high fibre, high carbohydrate dietary treatment

for diabetics was a decrease in fasting plasma glucose levels to an extent that the patients were able to reduce their insulin dosage (Anderson & Ward, 1979). These authors concluded that high carbohydrate/high fibre diets would benefit diabetics but the mechanism remained unclear.

The active component of these high fibre, high carbohydrate diets could be SCFA. This possibility has not being fully addressed scientifically in monogastrics. Todesco et al (1991) fed healthy subjects a bread supplement made with 2.7g/50g serving of sodium propionate for 7 days. A OGTT revealed that the propionate bread had decreased the area under the glucose curve by 38.0 ± 6.8% and the peak in plasma glucose was reduced by 10.5 ± 2.0% (Todesco et al, 1991). These results indicated that a high concentration of propionate in the diet is associated with improved glucose tolerance.

The influence of SCFA on insulin secretion has been more adequately addressed in ruminants. Mann & Boda (1967) examined the impact of acute SCFA infusion on insulin secretion of sheep. Propionic and butyric acid (2.5mmol/kg) were insulin secretagogues independent of glucose concentration (Mann & Boda, 1967). Similarly, jugular infusions of propionic acid (0.025mmol/kg/min) into sheep

increased plasma insulin concentrations after only 5 minutes (Horino et al,1968) whereas butyric acid was even more insulinogenic with peak insulin concentrations 1.8 fold greater than that of propionic acid. Brockman (1990) measured the effect of insulin on the utilization of propionate in gluconeogensis in sheep. Insulin was infused (2.40pmol/h) for 150 minutes and uptake of ^{14}C propionate was recorded. Endogenous glucose production decreased 30% but the incorporation of other glucose precursors decreased 30-50% and the utilization of propionate had decreased only 6% from control values (Brockman, 1990). Brockman (1991) concluded from these results that insulin differentially affected the rate at which glucose precursors are incorporated into glucose in sheep.

High fibre, high carbohydrate diets improved the glucose tolerance in humans and other monogastrics (Miranda & Horwitz, 1978; Kiehm et al, 1976). It has also been shown that SCFA have a glucose independent effect on plasma insulin concentrations in ruminants. It remains unknown if SCFA affect glucose disposal in monogastrics.

6 EXPERIMENTAL APPROACH

6.1 PIG AS A MODEL

The use of the porcine model in diabetes research is well recognized. A porcine model displaying glucose tolerance or intolerance similar to humans has been selectively bred at Colorado State University to aid in diabetic research (Phillips et al, 1982). The swine was chosen for this genetic development because of its pre-existing cardiovascular and metabolic similarities to humans. When exposed to a diet high in fat and simple carbohydrates, the pig also demonstrates impaired tolerance to a glucose load (Phillips et al, 1982). The sow is susceptible to both abnormal glucose tolerance during pregnancy (Schaffer et al, 1988) and to obesity given ad libitum feeding (Phillips et al, 1982). These metabolic responses are very similar to what can be observed in humans. The pig also demonstrates diabetes-related micro and macroangiopathies similar to those reported in humans (Phillips et al, 1982).

The swine model has also been used extensively to study the metabolic and gastrointestinal effects of dietary fibre. Rainbird and Low (1986a&b) found that the addition of guar to the pig diet slowed gastric emptying and increased

the viscosity of the digesta in the same manner as in humans (Rainbird & Low, 1986a&b). The post-prandial glucose profile of the pig when given guar with their diet resembled that seen in humans (Rainbird & Low, 1986b).

Pigs are capable of colonic fermentation of large amounts of dietary fibre (Low, 1985; Stanogias & Pearce, 1985). A high fibre diet causes proliferation of colonic bacteria (Low, 1985) and the proportions (Sambrook et al, 1979 : Cummings et al, 1981) and total amounts of SCFA produced in the pig colon are affected by the type and the level of fibre in the diet as it is with humans (Stanogias & Pearce, 1985). Pigs have a longer colonic transit time (50 hours)(Van Soest et al, 1982) than humans. Therefore, the pig has a greater opportunity to absorb SCFA. This is seen in work by Giusi et al (1985) where results showed 15-30% of porcine basal energy requirement could be obtained from SCFA. The rate of SCFA absorption in humans and pigs similar, 6-12μmol/cm^2/L/hour (McNeil et al, 1978) vs 8-10μmol/cm^2/hour (Argenzio et al, 1974).

Finally the porcine model has practical advantages. A 200 minute intra-venous glucose tolerance test requires an animal model that can have sufficient blood removed for glucose and insulin determinations. The pig model enables this to be done easily.

6.2 ROUTE OF DELIVERY

One of the difficulties associated with measuring SCFA delivery to the liver is that the gut metabolizes and utilizes a large portion of the SCFA produced before an accurate measure can be made. Therefore, to study the effects of SCFA on glucose tolerance an infusion technique was chosen which bypassed the gut to deliver known quantities of SCFA directly into the portal vein.

Examining the SCFA utilization in swine, Yen et al (1991) reported total SCFA concentrations of .01mmol/L in the portal circulation. Drochner et al (1984b) reported similar concentrations 2-12 hours post-prandially. The SCFA concentrations chosen for infusion in this study were based on the results of Yen et al (1991) and Drochner et al (1984b). Each SCFA was infused to deliver 0.01mmol SCFA/kg/min. This is the approximate value that would be expected post-prandially in portal circulation for propionic acid (9.6mmol/kg/24hr), higher than physiological concentrations for butyric acid (4.2mmol/kg/24hr) and less than is commonly seen for acetic acid (21.6mmol/kg/24hr) (Drochner et al, 1984b; Yen et al, 1991). Because the individual effects of each SCFA were to be compared, solutions were infused at a constant concentration per

kilogram. The experimental animals of this study were therefore receiving 14.4mmol SCFA/kg/24hr.

To deliver 0.01mmol SCFA/kg/min, the pump was set to deliver at a flow rate of 0.32mL/min and the SCFA concentration were adjusted according to pig weight.

7 **HYPOTHESIS:** High fibre diets are beneficial in the management of diabetes mellitus because SCFA production and absorption is increased which improves glucose regulation. Thus the infusion of short chain fatty acids into the portal vein of the porcine model will decrease fasting insulin, glucagon, free fatty acids and glucose levels and increase insulin sensitivity to an intravenous load of glucose.

8 **OBJECTIVE:**

1) To use the porcine model to determine the effect of portal infusions of SCFA on glucose tolerance and insulin sensitivity in monogastrics.

II. METHODOLOGY

1 ANIMALS

Six gilts with initial weights ranging from 16 to 22.5 kg (mean = 19.75 kg) were obtained from the Swine Unit at the University of Alberta Research Farm. The animals were housed individually in metabolic crates in the Laird McElroy Environmental and Metabolic Centre at the University of Alberta Research Farm. The animal room was maintained at a constant temperature (27°C). A 12 hour light (8:00am-8:00pm)/dark cycle was adopted. The animals were fed twice daily. They received 800g of feed between 8:00am and 8:30am and another 800g between 5:30pm and 6:00pm. Orts were removed from the feed bin and weighed before fresh feed was added. The animals had access to water ad libitum. All aspects of the experiment were approved by the University of Alberta Animal Policy and Welfare Committee.

1.1 EXPERIMENTAL DIET

The experimental diet was prepared at the Feed Mill at the University of Alberta Research Farm. Composition of the

TABLE II-1

Experimental Diet Composition

EXPERIMENTAL DIET COMPOSITION[1]	
	% AS FED
soyabean meal	12.00
wheat (Red Winter Hard)	44.00
dehulled oats	40.245
calcium chloride	1.25
dicalcium phosphate	.75
salt (NaCl)	.50
vitamin-mineral premix	1.00
choline chloride	.055
lysine HCl	.20
TOTAL	100.00

[1] the experimental diet provides 17.2% of energy through protein and 3.3% through fat.

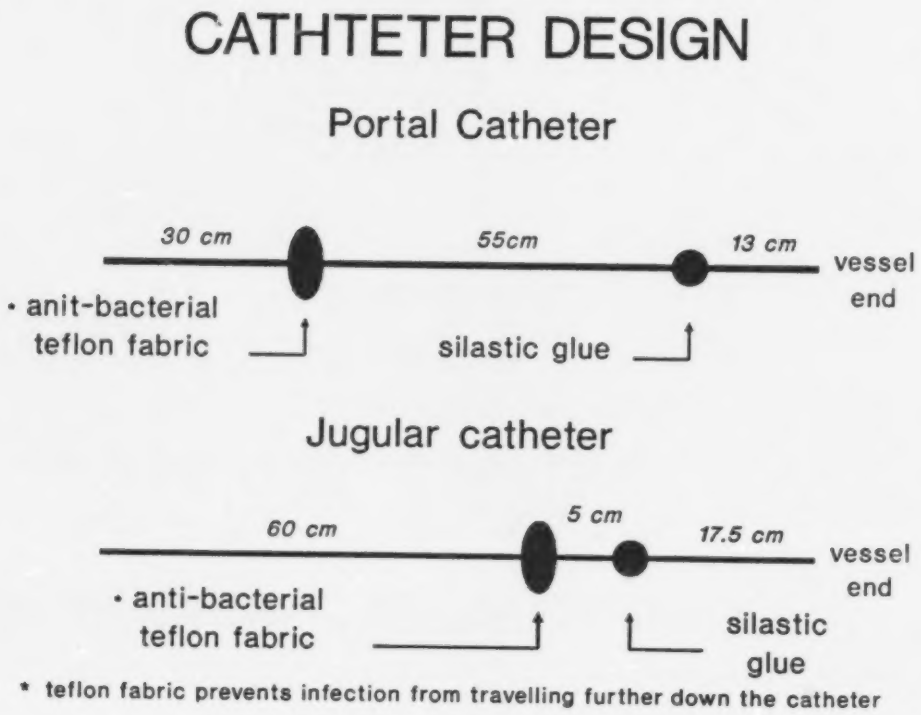
experimental diet is presented in Table II-1. This diet exceeds National Research Council's energy and nutrient requirements of pigs of this size and age.

1.2 SURGERY

The animals were fasted 24 hours prior to surgery. Approximately 30 minutes before surgery the pigs were sedated with Atravet (.56-1.110g/kg body weight) (acepromazine maleate 10mg/Ml, Ayerst). Surgery was performed under standard sterile conditions at the University of Alberta Research Farm. Halothane (2%) at 2.5-3L O_2/min was used to anaesthetize the animals. The pig was positioned on its left side. A small incision (2.5 cm) was made in the skin along the animal's spine flush with the bottom rib. Using large forceps, the portal catheter (2.64mm ID x 4.88mm OD Dow Corning, Midland Michigan USA) was inserted into the incision opening and down the right side of the animal between the skin and muscle tissue until it was just below the curvature of the rib cage. The catheter was left in this subcutaneous pocket to be retrieved from inside the abdominal cavity. The dorsal end of the catheter was exteriorized via a puncture incision approximately 1 cm from the original incision which was sutured with a continuous suture of 2/0 Dexon (Davis and Geek; Montreal, Quebec). Figure II-1 shows portal catheter size and construction.

FIGURE II-1

Portal and Jugular Catheter Design



The pig was repositioned onto its back. A laparotomy was performed along the linea alba. The peritoneal cavity was snipped and the muscular wall bluntly dissected to retrieve the portal catheter from the subcutaneous pocket into the peritoneal cavity. A 2-3cm length of mesentery vessel draining into the portal vein was isolated with two stay sutures (2/0 Dexon, Davis & Geck; Montreal, Quebec). The distal stay suture was ligated and tension applied to the proximal stay suture. A small incision was made in the vein and the silastic portal catheter was introduced into the vessel with a catheter introducer towards the liver for 13 cm (Becton Dickson, Rutherford New Jersey, USA). Both stay sutures were tied around the catheter to secure it. The intestine was organized into its original orientation and sterile saline (100-200mL) was added to the abdominal cavity. Prior to the closure of the incision terramycin 100mg/ml (.067ml/kg BW) (oxytetracycliune, Pfizer) was applied to the surgical sites in the peritoneal cavity. The intestinal incision was closed using a two layer closure. The muscle was sutured with interrupted vertical mattress sutures using 2/0 silk. The outer skin was closed with a continuous box suture of 2/0 Dexon. A blunted needle (Becton Dickson 18 G, 1") was inserted into the dorsal end of the catheter and wrapped with Tygon (3.18mm I.D. x 6.35mm O.D. & 6.4mm I.D. x 9.5mm O.D. Fisher Chemical: Pittsburgh,

Pen. USA) and taped to prevent perforation of the silastic catheter by the blunted needle. A male luer lock (Meditron, University of Alberta Hospital Materials Management, Edmonton, Alberta) was fitted onto the needle.

The jugular was then catheterized. A 6-8cm incision was made in the jugular area of the left side of the neck. The jugular vessel was isolated with two stay sutures (0/0 silk, Davis & Geck; Montreal, Quebec) and ligated at the proximal suture. An incision was made into the vein and a silastic catheter (1.57mm ID x 2.41mm OD)(Dow Corning, Midland Michigan USA) was introduced into the vessel using a catheter introducer and extended towards the heart for approximately 5cm. The stays were then tied around the catheter to hold it securely in the vessel. Figure II-1 shows jugular size and construction. Prior to closure Terramycin 100mg/ml (.067ml/kg BW) (oxytetracycline, Pfizer) was applied to the surgical sites inside the neck cavity. The neck incision was closed using a continuous stitch with 2/0 Dexon. The catheter was exteriorized on the back of the animals' neck between the shoulder blades using a trocar. A blunted needle (Hamilton 14 G 1/2) and male luer lock were inserted onto the exteriorized end of the catheter and secured with Tygon and tape in the same fashion as with the portal catheter. The catheter and needle were held in place

between the animals shoulder blades by a Whirl-pak and surgical tape.

Immediately post-operatively, .029ml/kg body weight Derapen-C (with Dihydrostreptonycin, Ayerst) was given intra-muscularly for long term antibiotic therapy.

1.3 RECOVERY

The pigs were returned to their metabolic crates immediately after surgery. They were kept under heat lamps for 12-24 hours. Food was withheld until the following morning at which time a small feeding (200-300g) was given. The feeding size was increased slowly to the full 1600g/day as the animal displayed an increase in appetite. After 3 days the animals were consuming a mean of 1328g/day (range; 1200-1456g). During the first 3 post-operative days the animals were given 500mg of Penbritin by intra-venous (ampicillin sodium) (250mg/ml dose:25-50mg/kg body weight, Ayerst) daily to minimize infection and decrease the occurrence of peritonitis.

During preliminary experiments clotting began to occur in some portal and jugular lines after 2-3 weeks making it impossible to sample blood although infusions could be

continued. The development of thrombi in a fistulated portal vein is a common problem in long term studies (4-6 weeks)(Drochner et al, 1984a). The infusion of heparinized saline is a recognized preventative measure for the patency of the catheter (Drochner et al, 1984a). Therefore, to avoid the development of thrombi, continuous infusions of heparinized saline (4.4IU/L, .32ml/min) were started immediately post-operatively for both the jugular and portal catheters and continued throughout the experiment.

Heparin administration increases lipolysis by causing increased activation of lipoprotein lipase (Abbate et al, unpublished data). Therefore, the addition of Hepalean to the infusion solutions may have created false concentrations of plasma free fatty acids. However, the use of Hepalean was mandatory to maintain catheter patency for the full 5 weeks of the study and all animals received 4400U/day of Hepalean (4.4mL). The incorporation of heparin into an IVGTT protocol does not alter the insulin sensitivity estimates of the minimal model method (Abbate et al, unpublished data).

2 EXPERIMENTAL DESIGN

A randomized design was used. Each pig received a saline infusion (control) and 3 treatment infusions (acetic acid, propionic acid and butyric acid). At the completion of the experiment all six animals had received the control treatment, acetate and propionate, 5 of the animals had successfully completed the butyrate treatment. One animal was euthanized prematurely because of displacement of the portal catheter from the vessel into the peritoneal cavity and subsequent peritonitis.

3 INFUSIONS: CONTROL AND EXPERIMENTAL

Treatment infusions began on the seventh or ninth day after surgery. Solutions were delivered at 0.32mL/min (460mL/24 hours) with an Ismatec IPS (Model 7619-40 Cole-Parmer, Chicago, Ill) peristaltic pump. An intra-venous drip set (primary piggy-back IV, Ventisystems, Abbot Ireland Ltd) was inserted into the infusion bottles and run to the tygon pump lines (Ismatec 0.76mm ID, Cole-Parmer, Chicago, Ill). Attached to the pump lines and running to the animal was 5-6 meters of medical grade Tygon tubing (1.2mm ID x 3.5mm OD) (Fisher Scientific). A needle (Becton-Dickson 20 G 1") was inserted blunt end into the Tygon line and sharp end into the

catheter luer lock on the animal. This was secured with tape. The entire length of infusion line was assembled and gas autoclaved before the initiation of the infusion.

On the seventh day of continuous treatment infusion, an IVGTT was performed (Bergman et al, 1981b). The treatment infusion continued throughout the IVGTT. At the end of the test day a new treatment infusion was started.

Physiological saline (.9% NaCl w/w)(Baxter: Toronto, Ont Canada) was obtained from the University of Alberta Hospital Materials Management and served as the control infusion and solvent to prepare the SCFA infusions. Pigs were weighed the day before the IVGTT so that the next treatment solution could be prepared to provide 0.01mmol SCFA/kg/min. The short chain fatty acid (SCFA) solutions were prepared under sterile conditions with physiological saline the day before the new treatment was introduced. The sodium salt of the three SCFA was formed by adding equimolar quantities of acetic acid (Aldrich Chemincal Co. Inc. Milwaukee, Wis), propionic acid (Aldrich Chemical Co. Inc. Milwaukee, Wis), or butyric acid (Aldrich Chemical Co. Inc. Milwaulee, Wis) with NaOH (BDH Inc. Toronto, Ont). The solutions were neutralized with NaOH to pH 7.0.

4 INTRA-VENOUS GLUCOSE TOLERANCE TEST

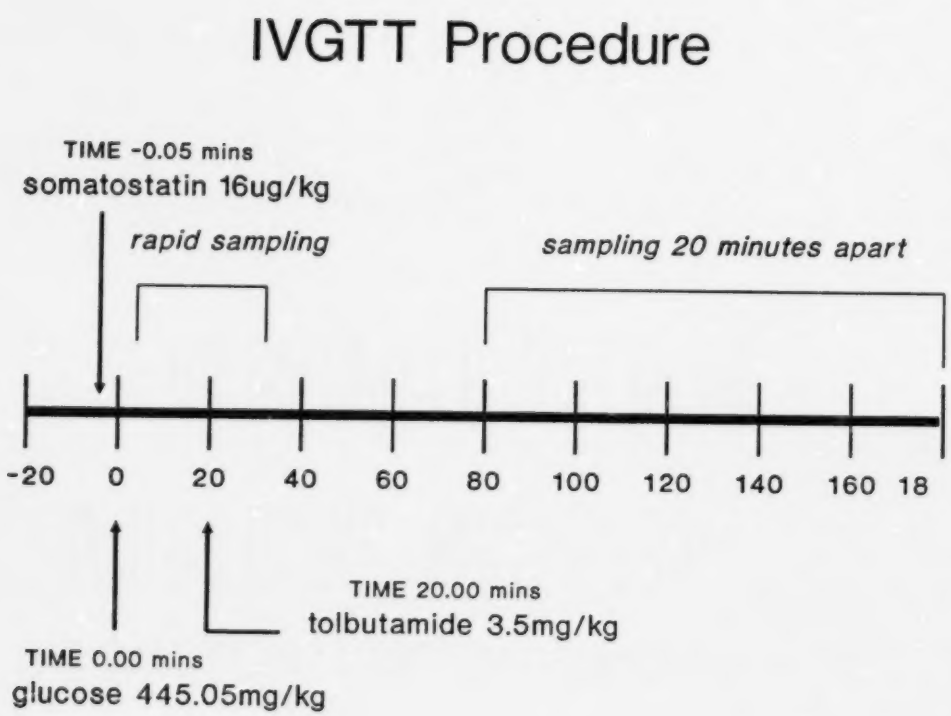
The IVGTT tests were scheduled for Day 7, 14, 21 and 28 of the experiment. Animals were not fed on the morning of the IVGTT. Blood samples (5mL) were taken from the jugular catheter at -20, -10, -5, -1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 21, 22, 23, 24, 25, 26, 28, 30, 35, 40, 50, 60, 70, 80, 100, 120, 140, 160, 180 minutes. The first blood sample (-20 min) was taken between 9:00-9:15am. Somatostatin (500ug/ml) (16ug/kg) (Bachem, Inc. Torrence California, USA) was injected at -0.5 minutes. Glucose (445.05mg/kg 50% dextrose, Baxter, Toronto, Ont. Canada) was infused over a period of 60 seconds at time 0. Tolbutamide (50mg/ml) (3.5mg/kg; compliments of Upjohn Co. Kalamazoo, MI) was injected at the 20 minute mark. Figure II-2 portrays a time line of the IVGTT procedure. At the completion of the IVGTT, the pig were given a prophylactic antibiotic (500mg Penbritin) via the jugular catheter.

5 BLOOD SAMPLE PREPARATION

A 5mL blood sample was taken at each timed interval. Approximately 3mL was placed in a polypropylene tube with 150μL of Hepalean (1000 USP Units/ml, Organon Teknika, Toronto, Canada) and 200μL of NaF (.05g/mL) (Fisher

FIGURE II-2

Time-line of Intravenous Glucose Tolerance Test



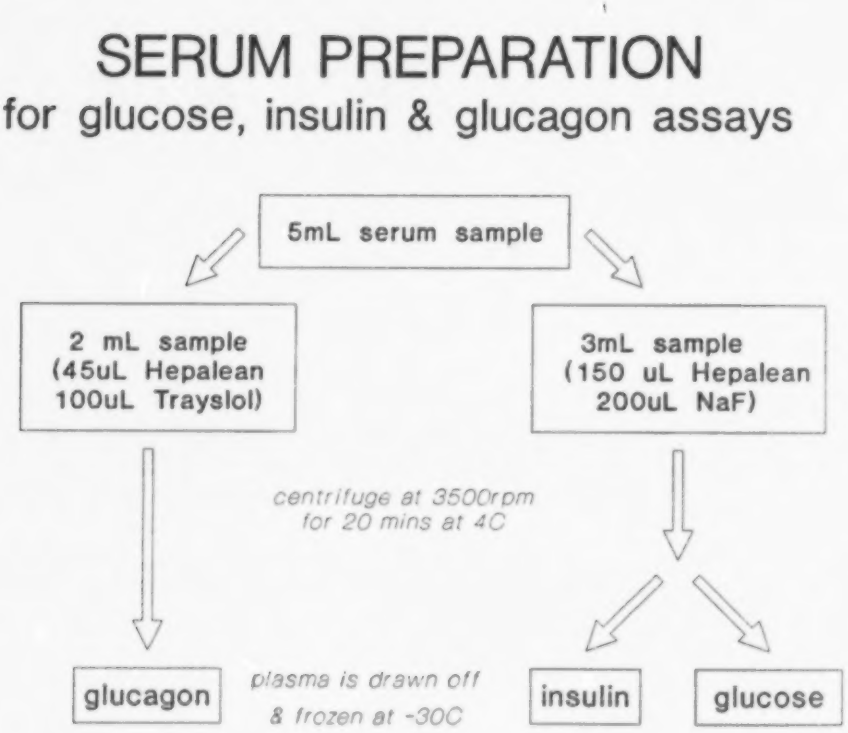
Scientific). The remaining 2mL of blood were placed in a separate polypropylene tube with 100μL of the aprotinin Trayslol (FBA Pharmaceuticals, New York) and 45μL of Hepalean. Blood samples were centrifuged for 20 minutes at 3500 rpm. The Trayslol plasma samples were removed with transfer pipettes (Fisher Scientific) and placed in clean, labelled polystyrene tubes. The NaF plasma samples were further split into two tubes to provide one for glucose analysis (1.5mL) and another for insulin determination (1.5mL). The Trayslol plasma was used for glucagon determination. This procedure is outlined in Figure II-3 All blood and plasma samples were kept on ice until frozen at -30°C.

5.1 GLUCOSE DETERMINATION

Glucose was analyzed using a Yellow Springs Instruments Enzymatic Glucose Analysizer (Model 27, Yellow Springs, OH). Plasma samples were centrifuged (MSE Centrifuge, John's Scientific Inc. Canada) for 5 minutes at 13,000 rpm prior to analysis. Replicates were analyzed until results were within .11 mmol and then the mean was calculated.

FIGURE II-3

Procedure of Serum Preparation for Assay



5.2 INSULIN & GLUCAGON DETERMINATION

Glucagon and insulin concentrations were measured at the Muttart Diabetes Research Centre, University of Alberta. Insulin concentrations were determined by the radioimmunoassay kit produced by Pharmacia Insulin RIA 100 (Pharmacia Diagnostics, Uppsala Sweden).Glucagon concentrations were determined using a double antibody radioimmunoassay (Ensinck et al, 1972 & Nonaka & Foa, 1970). After the plasma was preincubated with anti-glucagon anti-serum (rabbit, beef, pork glucagon: University of Texas), ^{125}I glucagon (Amersham), and non-labelled glucagon (human glucagon standards: Eli-Lily) the bound antibody-antigen complex was precipitated through the use of a carrier IgG (normal rabbit serum: MILAB, CedarLane) and an antibody to the carrier (second antibody: goat-anti rabbit immunoglobin: MILAB, CedarLane). Separation of the bound glucagon from the free was achieved by centrifugation. The anti-body precipitate was then counted on a gamma counter. Glucagon plasma concentrations were interpolated from standard curves.

5.3 FREE FATTY ACID DETERMINATION

The third fasting plasma sample from each IVGTT (-5 min) was used to determine free fatty acid concentrations using a methodology described by Miles et al (1983). A stock solution of 0.08 mol/L Tris buffer (pH 8.0) containing .6 mmol/L EDTA, 10mmol/L $MgCl_2$, and 0.1% (vol/vol) Triton X-100 was prepared and stored at 4°C. Stock solutions of ATP (100mmol/L), PEP (100mmol/L), NADH (40mmol/L) and coenzyme A (8mmol/L) were also prepared and aliquoted into daily amounts and frozen at -70°C until needed.

An oleate standard was prepared by dissolving sodium oleate in a 10mmol/L potassium phosphate buffer (pH 7.0) containing 4% human serum albumin (99% fatty acid free). The mixture was briefly sonicated and then frozen in aliquots at -70°C.

On a daily basis a buffer solution was prepared by adding NADH (47nmol/mL), ATP (840nmol/mL), PEP (970nmol/mL), myokinase (3.3U/mL), pyruvate kinase (1.5U/mL), lactic dehydrogenase (6U/mL) and acyl-CoA synthetase (1.5mU/Ml) to the stock Tris buffer.

Two-hundred microliters of stock standard were diluted in 50mL of 50mmol/L potassium phosphate buffer pH 7.0 to

produce a standard concentration of 0.8mmol/L. With further dilution, six more standards were prepared (0.6, 0.4, 0.3, 0.2, 0.1, 0.056mmol/L).

Forty microliters of each standard were pipetted in triplicate into 10x75mm disposable glass tubes. Twenty microliters of plasma were then pipetted in triplicate into separate tubes. Two milliliters of the Tris buffer solution were added to each tube and vortexed briefly. All tubes were then incubated for 10 minutes and the absorbance of the standards and plasma tubes were read at 340nm spectrophotometrically (Spetronic 20D, Milton Roy Co., USA). Coenzyme A (20μL) was then added to each tube which was briefly vortexed. The tubes were incubated for 75-90 minutes. Absorbances were read at 340nm and the difference between the first and second readings were indicative of NADH oxidation. Using the standard curve equation, the differences were calculated to determine the free fatty acid concentration (mmol/L).

5.4 DETERMINATION OF INSULIN SENSITIVITY & GLUCOSE TOLERANCE

Insulin sensitivities (S_I) were calculated using the Minimal Model method (Bergman et al, 1986) to analyze the

plasma glucose and insulin dynamics during the IVGTT. A second method which was used to evaluate the impact of SCFA of the metabolic handling of glucose was the calculation of the glucose tolerance (K_G). This value is determined by taking the natural log of the glucose concentrations and plotting it against time (6-35 mins). The K_G is the slope of that plot expressed as a percentage.

6 STATISTICAL ANALYSIS

A Statistical Analysis System (SAS Institute Inc, Cary, NC) procedure for general linear models (PROC GLM) was used for the analysis of variance on the fasting concentrations of glucose, insulin, glucagon and FFA. Because it can accommodate unequal numbers of observations and different sources of variance, PROC GLM was also used to determine significance between the feed intakes and the mean weight gain for both treatment and period. In addition to the PROC GLM analysis, significant effects were further explored by Duncan's multiple range test (Steele & Torrie, 1980).

PROC MEANS was employed to generate means and standard errors of the mean (SEM) for glucose, insulin and glucagon curves.

Repeated analysis of variance for the mean glucose, insulin and glucagon curves were performed in SAS. Because a repeated analysis will not be complete if missing values are present, a second analysis was run in SAS which incorporated all the assay values. There were no significant differences between the results of the repeated analysis of variance and the second analysis of variance, therefore, the results of the repeated analysis are valid.

Regression analyses to determine K_G were performed in Lotus 123. PROC GLM was used to determine effect of treatment on K_G and S_I.

For all tests, the p value to accept or reject a significant difference was set as a $p < 0.05$.

III. RESULTS

Infusion treatment did not significantly ($p>0.05$) affect feed intakes (Table III-1), or weight gain (Table III-2). There were however, period effects on feed intake. Feed intakes in the first (1294.1 g/day) and fourth (1341.5 g/day) periods were significantly lower than those in the second (1496.8 g/day) and third periods (1520.4 g/day) ($p<0.0001$). The decreased intake in the first period reflects the depressed post-operative appetite and the incomplete appetite recovery in the first treatment period. The reduced average feed consumption in the fourth and final experimental period is lowered because two animals began to go off feed a few days before the final IVGTT. The difference in feeding between the four periods did not significantly affect weight gains. Among periods however, the mean animal weight gains by period were 4.9 ± .6 kg, 4.8 ± .3 kg, 4.8 ± .5 kg and 5.9 ± .6 kg, respectively.

Fasting levels of glucose, insulin, glucagon and free fatty acids are presented in Table III-3. The fasting values for glucose, insulin and glucagon were not altered by the infusion of acetic, propionic or butyric acid relative to the experimental saline control. Fasting glucagon concentrations showed greater variability than did glucose or insulin

TABLE III-1

Summary of Feed Intake by Treatment

TREATMENT	MEAN FEED INTAKE (g) ± SEM
PROPIONIC ACID	1414.6 ± 39.2
BUTYRIC ACID	1445.3 ± 35.0
ACETIC ACID	1381.1 ± 63.3
SALINE (CONTROL)	1429.1 ± 37.8

TABLE III-2

Summary of Weight Change by Treatment

TREATMENT	WEIGHT CHANGE (kg) ± SEM
PROPIONIC ACID	5.1 ± .5
BUTYRIC ACID	5.6 ± .4
ACETIC ACID	4.9 ± .5
SALINE (CONTROL)	4.8 ± .7

TABLE III-3

Mean Fasting Levels of Glucose, Insulin,

Glucagon and Free Fatty Acids

TREATMENT	FASTING GLUCOSE mmol/L	FASTING INSULIN pmol/L	FASTING GLUCAGON ng/L	FASTING FFA mmol/L
PROPIONIC ACID	5.58 ± .35	36.6 ± .3.6	170.8 ± 4.9	.12 ± .1
BUTYRIC ACID	5.11 ± .07	35.4 ± 10.2	153.9 ± 7.7	.37 ± .2
ACETIC ACID	5.15 ± .20	30.6 ± 5.4	145.8 ± 17.7	.49 ± .2
SALINE (CONTROL)	5.55 ± .15	44.4 ± 11.4	169.6 ± 20.1	.26 ± .1

concentrations. Fasting free fatty acid concentrations were not affected by treatment.

Insulin sensitivity was not significantly influenced by SCFA infusion (Table III-4). Similarly, (K_G) was not significantly affected by SCFA infusion (Table III-5). Comparable trends were observed in glucose tolerance as with S_I; ie: the glucose tolerance was highest with saline (4.9 ± .6 min-1), followed by butyric acid (4.8 ± .5min $^{-1}$), propionic acid (3.9 ± .3 min^{-1}) and finally acetic acid (3.5 ± .5 min^{-1}). Insulin sensitivity was significantly influenced by period. When the S_I data was expressed as a percentage of the first period S_I value, the fourth period (44.9 ± 11.8%) was significantly different than the third (78.4 ± 12.7%), second (78.6 ± 14.2%) and first (100%) periods.

Repeated analysis of variance was used to analyze sequential treatment means for glucose (Figure III-1), insulin (Figure III-2) and glucagon (Figure III-3). The mean glucose curves were unaffected by SCFA infusion.

The mean insulin curves are found in Figure III-2. Treatment infusion did not affect mean insulin levels throughout the IVGTT at any time.

TABLE III-4

Mean Insulin Sensitivity by Treatment

TREATMENT	MEAN S_I ± SEM (min^{-1}/uU/mL)
PROPIONIC ACID	28.87 ± 8.31
BUTYRIC ACID	38.54 ± 7.91
ACETIC ACID	28.55 ± 11.42
SALINE (CONTROL)	41.46 ± 13.19

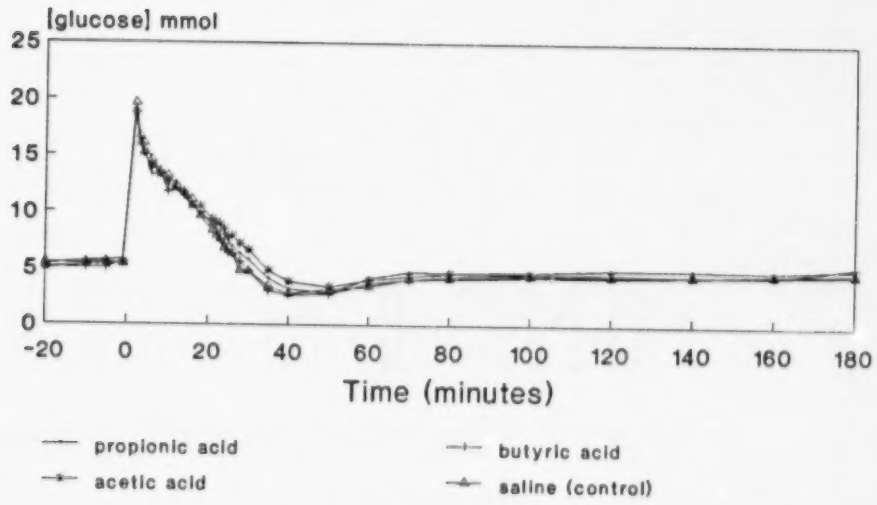
TABLE III-5

Mean Glucose Tolerance by Treatment

TREATMENT	MEAN K_G ± SEM (min^{-1})
PROPIONC ACID	3.8 ± .3
BUTYRIC ACID	4.7 ± .5
ACETIC ACID	3.5 ± .5
SALINE (CONTROL)	4.9 ± .6

FIGURE III-1

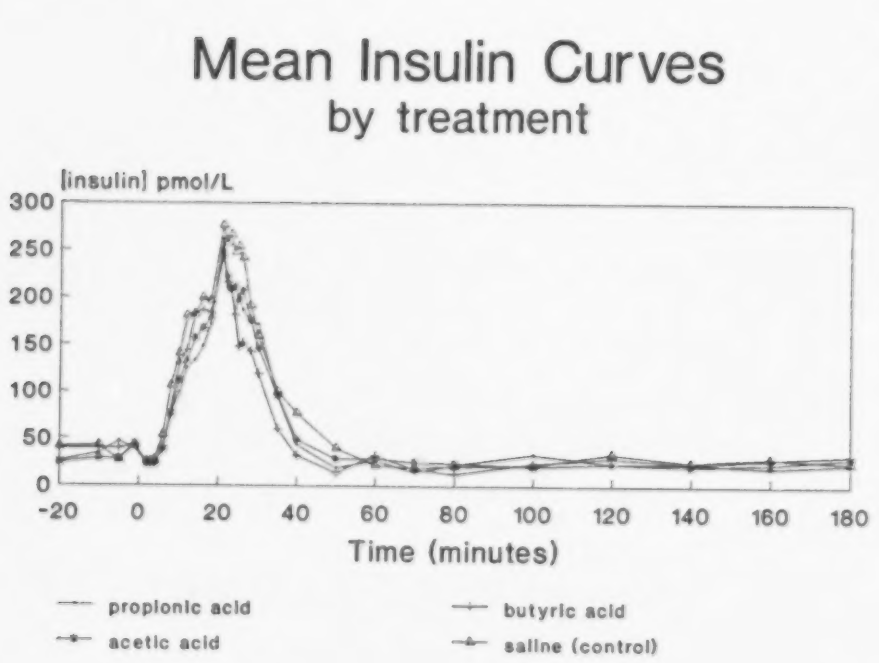
Mean Glucose Curves by Treatment



$p > 0.05$ among treatments within times

FIGURE III-2

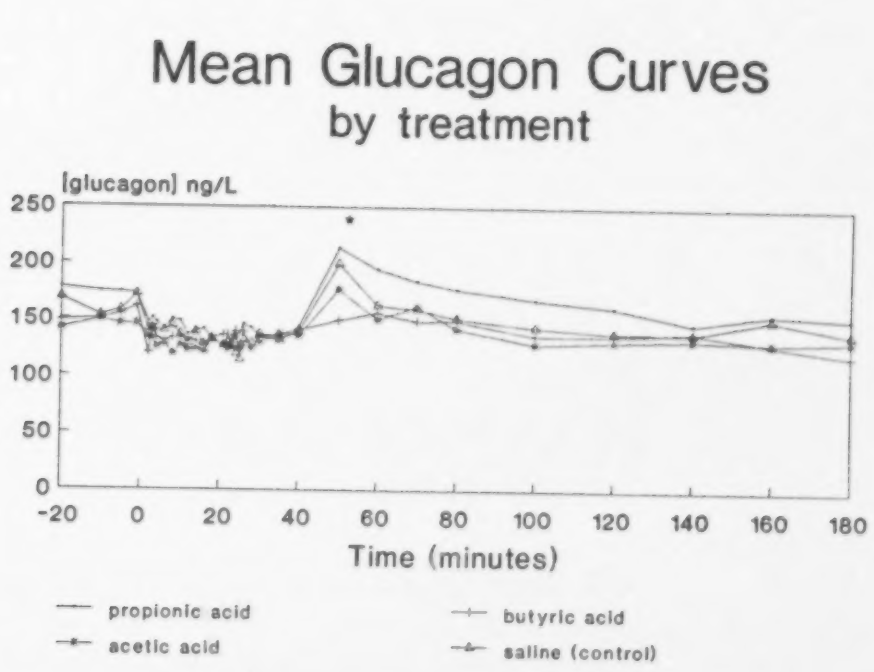
Mean Insulin Curves by Treatment



$p > 0.05$ among treatments within times

FIGURE III-3

Mean Glucagon Curves by Treatment



* $p < 0.05$ propionic versus butyric acid

A significant difference was observed between propionic acid versus butyric acid (Figure III-3) at 50 min after the glucose load. This peak in the glucagon curve is the only point which reached statistical significance ($p<0.05$).

IV. DISCUSSION

The objective of this experiment was to determine the effect of SCFA portal infusions on glucose tolerance and insulin sensitivity in a porcine model. An examination of the results clearly demonstrates that portal infusions of short chain fatty acids have no effect on the insulin sensitivity or the glucose tolerance in the porcine model.

Throughout the course of this study, the pigs increased their body mass by approximately 48%. An increase in adipose tissue has been seen to decrease insulin sensitivity (Bergman et al, 1981b; Anderson & Bryant, 1986) whereas an increase in muscle tissue will increase S_I. This study was designed to accommodate this expected growth by allocating the injections of glucose, somatostatin and tolbutamide on a per kilogram body weight basis. This assumes that the pigs weight gain had a characteristic and constant proportion of lean body mass and adipose tissue which may not be true. Although weight gain did not differ among periods the S_I was seen to significantly decrease ($p>.02$) as the study progressed from period 1 to 4. From the reduction in S_I it might be predicted that the weight gained between the first and fourth period was more adipose tissue

than muscle tissue.

The gilts used in this study were observed to be 6-7 times more insulin sensitive than humans (5.1 ± 1.3 min^{-1}/ μU/mL) (Bergman & Bowden 1981) and 4-5 times more sensitive than dogs (7.8 ± 0.7 min^{-1}/μU/mL) (Finegood et al, 1989). There was no improvement in the animals' sensitivity to insulin with the infusion of any of the SCFA treatments. The results of the S_I are supported by the calculated K_G values. The glucose tolerance of the pig (4.23 ± .5) is approximately two times greater than that of humans (1.8 ± .02min^{-1}) (Bergman et al, 1981b) and similar to the values seen in dogs (4.24 ± .44 min^{-1}) (Finegood et al, 1989). The treatment infusions did not lead to improved glucose tolerance or insulin sensitivity over the control. It is apparent from Tables III-4 and III-5 that there was marked variability between pigs in S_I and K_G.

Short chain fatty acid infusion did not significantly affect fasting levels of glucose or insulin (Table III-3). These results do not support human studies where fasting levels of glucose and insulin were seen to be decreased 4-12 hours after the consumption of fibre (Jenkins et al, 1980; Anderson & Ward, 1979; Pastors et al, 1991). These results do however support work performed by Horino et al (1968) who examined the effects of propionic acid on the plasma insulin response of pigs. No change in the animals' insulin response

was seen after a 20 minute infusion of propionic acid (.0250 mmol/kg/min). The results of this thesis found no apparent change in insulin response after a continuous seven day infusion of 0.01mmol SCFA/kg/min.

The infusion of SCFA did not induce a notable change in the fasting concentrations of glucagon nor free fatty acid (Table III-3).

There was wide variability in the fasting insulin concentrations between pigs. This study was run in the form of three blocks of two animals per block. The mean fasting insulin values in the first block of the experiment were significantly higher than the values seen in the second and third blocks ($p<0.02$). The glucose, glucagon and free fatty acids did not demonstrate a similar pattern. A random sampling of insulin serum samples from across all three of the blocks were run in a blinded assay to determine if this difference between the blocks was due to a methodological inaccuracy. The values were not different in this second analysis. Therefore we can conclude that the significant differences in insulin concentrations seen between the first block and the second and third is a true difference. This is an excellent example of the animal variation observed in this study.

circulation. These diets must therefore produce the observed positive consequence in some other manner.

Both colonic infusions of physiological concentrations of pectin (Koruda et al, 1986) and SCFA supplemented total parenteral diets (Koruda et al, 1988) have been shown to be hypertrophic to the small and large intestine of the rat. Butyrate is the principle mediator of the increase in colonic weight (Rombeau, 1988). An increase in intestinal mass represents an increase in the number of epithelial cells. It could be that an elevation in cell number increases glucose utilization by gut epithelial cells, decreasing the amount of oral glucose reaching portal circulation. In this study, the gastrointestinal tract was bypassed to minimize changes in gastrointestinal mass. Further, in this study the glucose bolus was infused intravenously via the jugular vein which would diminsh potenial effects of the gut on glucose tolerance. However, gut hypertrophy could be a mechanism whereby oral glucose tolerance improved in long term feeding studies like those of Kiehm et al (1976), Miranda & Horowitz (1978) and Anderson & Ward (1979).

Other possible explanations must also be considered in rationalizing differences observed in this study verus other longterm fibre studies. There is a significant increase in insulin response to a meal including protein versus one which

is composed only of carbohydrate (Hollenbeck & Coulston, 1991). As a result, the rise in post-prandial plasma glucose response is decreased. The protein content of the control diet of Anderson & Ward (1979) was 92g compared to 98g in the experimental diet. Differences are again seen when examining the protein content of control (110 ± 17g; 35.9) and test (106 ± 20g; 91.3) meals of Anderson et al (1991) and Wolever et al (1988) respectively. Since the test meals used in the aforementioned studies differed in protein content as well as in the fibre component, this may have influenced circulating insulin concentrations and in turn generated an erroneous estimate of glucose tolerance.

The amount and/or type of dietary fat present existing in a mixed diet will also influence the insulin, and ensuing plasma glucose response over and above that of protein (Hollenbeck & Coulston, 1991).

The fatty acid composition of the diet is a major factor influencing the fatty acid composition of cellular membranes. Innis & Clandinin (1981) reported that feeding rats a diet of canola oil for as little as 12 days alters cellular membrane composition compared to a crossover diet soyabean oil.

The insulin receptor is embedded in the plasma membrane lipid bilayer. Therefore, the insulin receptor (Ginsberg et al, 1981) and its function (Sandra et al, 1984) are also affected by changes in the lipid environment. MacDonald et al (1991) examined the impact of fibre on insulin binding in the GIT. Their results suggest that the insulin receptors located within the mucosal wall are modified by the ingestion of dietary fibre. A oat bran or wheat bran diet was associated with changes in the insulin receptor binding and autophosphorylation in the jejunum of rats (MacDonald et al, 1991). Field et al (1988) explored changes in the insulin receptor function in adipocytes of diabetic and non-diabetic rats. At all insulin concentrations explored, the adipocytes of the non-diabetic rats bound more insulin on the high P/S ratio diets (P/S = 1.0) than the low P/S ratio (P/S = 0.25) in a linear fashion (Field et al, 1988 & 1989). At high insulin concentrations (100ng/mL and 1000ng/mL) glucose transport was increased on the high P/S ratio diet. The dietary fat also improved the % insulin bound and the glucose oxidation of the diabetic animals (Field et al, 1989). From the examination of these studies, it can be inferred that the fatty acid composition of diet, can have positive and negative repercussions on the tissue disposal of glucose and the insulin response to a glucose challenge.

In most of the cited studies showing an improvement in glucose tolerance on high fibre diets, the increase in the carbohydrate of the experimental diet versus control was due to the replacement of fat. Anderson & Ward (1979) had an total difference of 56g of fat between the control and experimental diets. There were large discrepancies between the amounts of saturated (21g) fats and monosaturated (34g) fats. This pattern is repeated in the work of Anderson et al (1991). In this dietary comparison there were also notable differences in the PUFA content of the diets. Many of the experiments cited neglected to outline the profiles of the fatty acids in the contrasted diets and therefore comparison cannot be made (Miranda & Horwitz, 1979; Kiehm et al, 1976; Pastors et al, 1991; Wolever et al, 1988). Differences in the amount and type of fats present in the diets could have affected the glucose disposal and insulin sensitivity values reported in the aforementioned studies rather than the fibre intakes perse.

In conclusion, results from this trial do not suggest that the portal infusion of propionic, butyric or acetic acid cause improvement in glucose tolerance or fasting levels of glucose, insulin, glucagon or free fatty acids. Therefore, the improved insulin sensitivity seen in subjects on long term high fibre high carbohydrate diets cannot be attributed

to the increased concentrations of SCFA in the portal circulation.

The next step in research in this area should focus on the absorption and metabolism of SCFA in the lumen of the large intestine. This would allow for the inclusion of the role of the gut epithelium in glucose tolerance. The beneficial effects of fibre do not seem to be due to the presence of SCFA in portal circulation, therefore steps preceding this should be examined. If SCFA were infused into the colonic lumen the role of the gut in glucose tolerance and insulin sensitivity could be examined.

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